Title (en)

A METHOD FOR IN VITRO MOLECULAR EVOLUTION OF PROTEIN FUNCTION

Title (de)

EINE METHODE ZUR IN VITRO MOLEKULAREN EVOLUTION DER PROTEINFUNKTION

Title (fr)

METHODE DE DEVELOPPEMENT MOLECULAIRE IN VITRO D'UNE FONCTION DE PROTEINE

Publication

EP 1504098 A2 20050209 (EN)

Application

EP 03722867 A 20030516

Priority

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- US 32119502 A 20021217

Abstract (en)

[origin: US7262012B2] A method for in vitro molecular evolution of protein function The invention provides a method for generating a polynucleotide sequence or population of sequences from parent single-stranded polynucleotide sequences encoding one or more protein motifs, comprising the steps of (a) providing a first population of single-stranded polynucleotide molecules and a second population of single-stranded polynucleotide molecules, the first and second populations together constituting plus and minus strands of parent polynucleotide sequences, (b) carrying out a reaction for digesting the first and second populations of single-stranded polynucleotide molecules with an exonuclease to generate corresponding populations of single-stranded polynucleotide fragments, (c) contacting said fragments generated from the plus strands with fragments generated from the minus strands and optionally, adding primer sequences that anneal to the 3' and 5'ends of at least one of the parent polynucleotides under annealing conditions, and (d) amplifying the fragments that anneal to each other to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics as compared to the one or more protein motifs encoded by said parent polynucleotides, wherein, in step (b), at least one parameter of the reaction used for digestion of the first population of single-stranded polynucleotide molecules is different from the equivalent parameter(s) used in the reaction for digestion of the second population of single-stranded polynucleotide molecules. Preferably, the reaction parameter is selected from exonuclease type, exonuclease concentration, reaction volume, duration of the digestion reaction, temperature of the reaction mixture, pH of the reaction mixture, length of parent single-stranded polynucleotide sequences, amount of single-stranded polynucleotide molecules and buffer composition of the reaction mixture.

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C12N 15/10; C12Q 1/68

IPC 8 full level

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